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Fungi on Dragon Fruit in Loei Province, Thailand and the Ability of *Bipolaris cactivora* to Cause Post-harvest Fruit Rot

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Abstract

Dragon fruit [*Hylocereus undatus* (Haworth) Britton & Rose] grown in Loei province is one of dragon fruit cultivars with high quality and rich of sweetness compared to the fruits from other areas. There were some fungal infections observed in post-harvested diseases caused serious damage on the fruits leading to serious damages to the produces. Therefore, Fungi were isolated from three infected parts of the plants e.g. stems, flowers and fruits, in relevant to the causative pathogens. Two fungal species, *Alternaria* sp. and *Phomopsis* sp. were found on the stems. Six species, *Bipolaris cactivora*, *Cladosporium cucumerinum*, *Fusarium* sp., *Alternaria* sp. and *Colletotrichum gloeosporiodes* were obtained from rotten flowers. From the fruits, three fungi, *B. cactivora*, *C. gloeosporiodes* and *Rhizopus stolonifer* were isolated. The most frequently species isolated from the fruits and some epidemiological aspects of the pathogen were studied in details.
Keywords: *Drechslera, Hylocereus undatus, fitness aspects, infection processes*

1. Introduction

Hylocereus undatus (Haworth) Britton & Rose is a tropical climbing cactus, which is also known as pitaya, dragon fruit, strawberry pear and night blooming cereus. This fruit is first known in Mexico and South America. Currently, this plant has been commercially cultivated in Southeast Asian countries; specifically Malaysia, Thailand, Vietnam including Taiwan (1). This fruit has been reported to harbor many fungal species after harvesting such as *Bipolaris cactivora* (Petr.) Alcorn, *Botryosphaeria dothidea* (Moug. ex Fr.) Ces. & De Not., *Colletotrichum capsici* (Syd.) E.J. Butler & Bisby, *C. gloeosporiodes* (Penz.) Penz. and Sacc., *C. truncatum* (Schwein.) Andrus & W.D. Moore, *Curvularia lunata* (Wakker) Boedijn, *Fusarium semitectum* Berk. & Ravenel, *Gilbertella persicaria* (E.D. Eddy) Hesselt. and *Monilinia fructicola* (Wint.) Honey. (1–5)

The dragon fruit plants were found to be infected by different fungi in different countries. In Mexico, in small-scale areas where *H. undatus* were grown, the plants were indicated with stem spots. The symptoms began with small chlorotic dots caused by *B. dothidea* (6). In Malaysia and Thailand, *Collectotrichum* species were commonly pathogenic on this plant. *C. gloeosporiodes* is a causing agent of anthracnose disease on both stem and fruit (1). The symptoms can be indicated by reddish-brown color with chlorotic haloes lesions on infected stem and fruit. However, it is not different from widely seen rot disease caused by *B. cactivora* previously reported on stems and fruits of dragon fruit (7,8). Even though this pathogen does not seriously damage mature stems, it is highly influential to the post-harvest fruits (8).

In Thailand, *B. cactivora* is usually discovered on dragon fruit according to Athipunyakom et al. (2009), reported about fruit rot of dragon fruit caused by this pathogen (9). It has been also widely found in Japan, Israel, South-Florida, Vietnam and Europe (7,8,11,12,13). This causal pathogenic agent was not only identified from the dragon fruits but also distributed in cactus growing areas as a fungus causing the stem rot disease (14).

The identification and pathogenicity test of this pathogen was necessary to examine the epidemiological aspect of *B. cactivora* as the detailed report associated with the pathogen on dragon fruits in Thailand particularly from Loei province was rare. Therefore, this study targeted to investigate the development of the fungus *B. cactivora* in dragon fruits during storage and to observe the fungal life cycle for its epidemiological significance to the fruits.

2. Materials and Methods

Dragon fruit samples. The infected stems, rotten flowers and fruits of dragon fruit (*Hylocereus undatus*) were taken from Dan Sai, Pak Chom and Phu Ruea district Loei province, Thailand.

Fungal isolation. The disease plant parts were cut at margin of lesions into small pieces (5 mm x 5 mm), immersed with 70% ethanol for 30s and in 2% hypochlorous for 90s, then washed with sterile distilled water, and dried on an uncontaminated bench. Sterilized of surface lesion tissues then placed on PDA plates and incubate at 25 ºC. Hyphal tips were aseptically transferred to new PDA. The rotten flowers were observed for the prospective fungal pathogens under microscope because the flowers might be the source of the fungal inoculum, which later caused the rot disease on the fruits.

Morphology study. In order to observe conidia and conidiophores, the fungal mycelia were inoculated on surface-sterilized dragon fruits using 10% Clorox for 5 mins and incubated at 25±1 °C for 4 days. Conidia and conidiophores were mounted in distilled water and observed under a Carl Zeiss Axioplan2 compound light microscope. Conidial size measurements were taken from the length and width part of each conidium. Conidial length and width were measured using Axiovision Rel. v. 4.8.2 software (Carl Zeiss Microscopy, Thornwood, NY, USA). One hundred conidia were measured and calculated by the 5^{th} and 95^{th} percentiles for all measurements of conidia then follow by mean, minimum, maximum, and standard deviation (15,16).

Pathogenicity test of *Bipolaris cactivora.* The fruit surface was sterilized

using 10% Clorox for 5 min, then washed with sterile distilled water for 5 min twice, and air-dried on a clean bench. Mycelial plugs (8 mm in diameter) from the edge of growing cultures on PDA were placed on the center of fresh PDA and incubated for 7 days. After that, 7-day-old mycelial plugs from PDA were placed on the surface of the fruits upside down to let the mycelium attach to the fruit surface and kept in moist chambers for 4-7 days with room temperature (25 ± 1ºC).

Epidemiology Study of *Bipolaris cactivora*

Incubation period. The period between exposure of spore inoculation to an infection and the first appearance of the symptoms.

Latent period. After inoculation on the fruits, the time for producing the first mature spores were hourly recorded.

Lesion expansion. Diameters of symptom were measured from the first appearance of the symptom after every 6 hours until 42 hours.

Sporulation ability. After measuring the diameters of the lesions on the fruits, the fruits were kept until 96 hours then the spores were counted. The diseased areas on the fruits were cut then suspended in 10 ml sterile distilled water containing one drop of 20% Tween then agitated by using C10 platform shaker (New Brunswick Scientific, Edison, NJ, USA) for 10 minutes with 120 rpm to separate the spores from the fruit tissues. Lastly using pipet and Haemocytometer, the number of conidia was determined.

Statistical analysis. All experiments were conducted with four replications. The 5th and 95th percentiles used calculated using Microsoft Excel 2013. The first time of symptom, conidia and number of conidia was analyzed for one way ANOVA with Duncan multiple range test by using Statistix8 Program. For the correlation of time and fungal colonies, it was also calculated using the same program.

3. Results and discussion

Fungal pathogens of dragon fruit

It was expected that the pathogenic fungi resided in different parts of the plants prior to the fruit development. Thus, fungi were isolated from three parts of the plant tissues, stems, flowers and fruits for causative pathogens. *Alternaria* sp. and *Phomopsis* sp. were found on the stems. From rotten flowers, there were *B. cactivora*, *C. cucumerinum*, *Fusarium* sp., *Alternaria* sp. and *C. gloeosporiodes* (Figure 1) From the fruits, *B. cactivora*, *C. gloeosporiodes* and *Rhizopus stolonifer* were obtained (Table 1). These fungal pathogens have been significantly influential to the post-harvest fruit rot in many countries. *Alternaria* sp. was another fungus detected from the fruits causing a disease in post-harvest fruits (17). It is also a fungal pathogen leading to a serious damage in post-harvest dragon fruits from 16 countries imported into Shianghai (18). In Thailand, there are records reporting that *Phomopsis* sp. and *A. alternata* are from flowers of dragon fruit (9) and *Phomopsis* sp. is a primary pathogen on sunflower (19). Furthermore, Athipunyakom et al. (2009) and Sornvilai et al. (2012) who found that *Dothiorella dominicana*, *C. gloeosporioides*, *C. capsici, and B. cactivora*, which are associated with fruit rot diseases, have been reported postharvest diseases of this plant. The anthracnose disease on the fruit caused by *C. gloeosporiodes* as shown in Figure 3

was additionally found. This similar case was found on the stem and fruits of the plants from Malaysia (1). Another pathogen found in the dragon fruits, *R. stolonifer,* it is one of the most harmful pathogen on post-harvest fruits causing the soft rot disease, which severely affects the fruit quality (20). The management of this pathogen is difficult as the pathogen is able to trigger the disease symptom in a short period eventually leading to the fruit decay (21).

The flowers of the dragon fruits were expected to be the first source of inoculum of fungal pathogens causing the disease on post-harvest dragon fruits. Therefore, according to the frequencies of the fungi from thirty rotten flowers, the numbers of observed fungal pathogens were recorded and it indicated that the most frequent found fungus was *B. cactivora*, 100% and *Cladosporium* sp. 100% followed by *Alternaria* sp. and *Fusarium* sp., 60% and 70% respectively and *C. gloeosporiodes* was only about 37%. Although the *B. cactivora* and *Cladosporium* sp. were found in all flowers, the significant pathogen causing the rot disease in the fruits was only *B. cactivora* according to this research. Thus, this fungus was subjected to the epidemiological study.

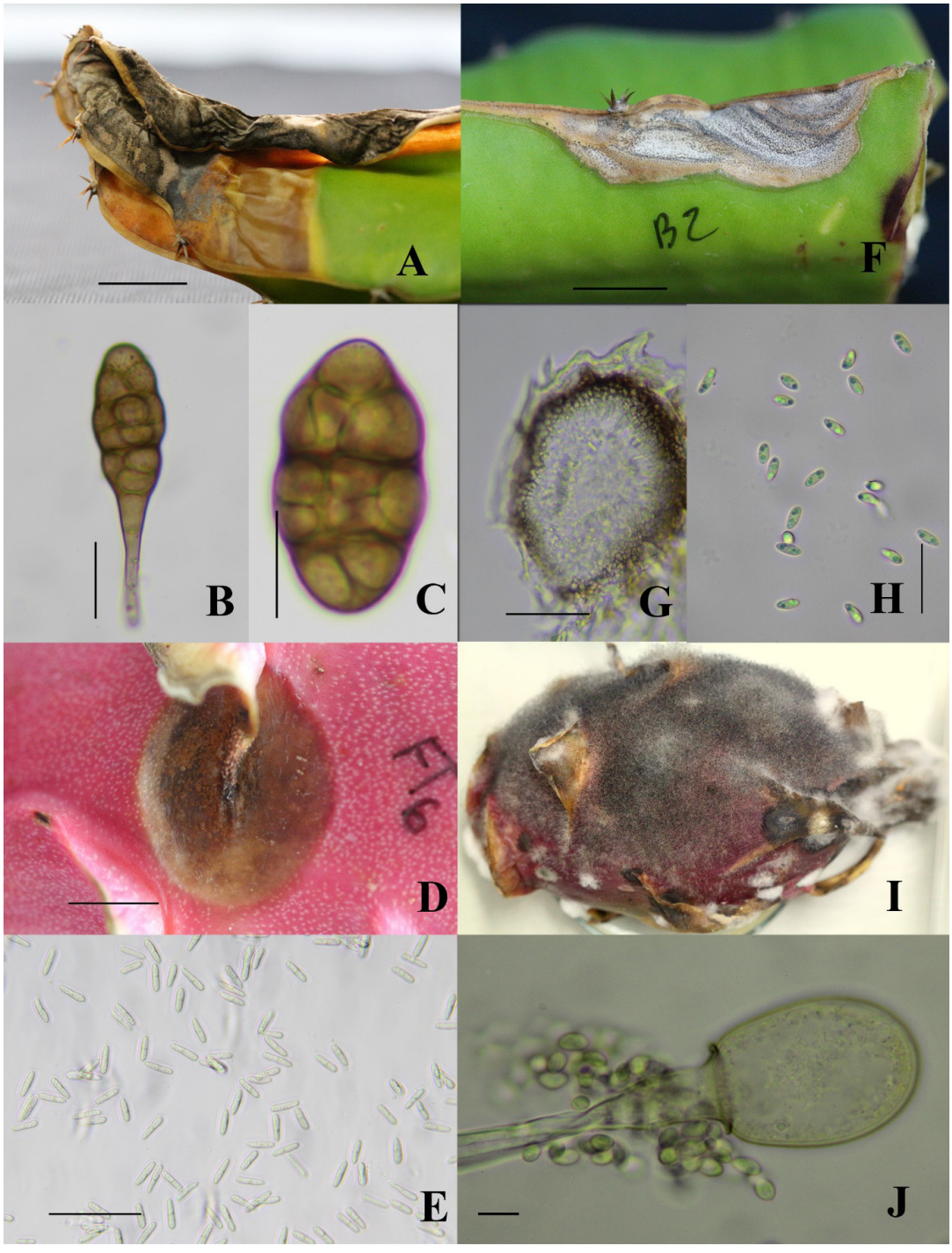

Figure 1. The symptoms of dragon fruit caused by fungal diseases, **A-C:** The infected stem rot caused by *Alternaris* sp. and conidia, **D-E:** The fruit rot symptom caused by *Colletotrichum gloeosporiodes* with mass of conidia, **F-H:** The symptom of *Phomopsis* sp. with gray-black spot and pycnidia with emerging mass of conidia**, I-J:** Soft rot symptom caused by *Rhizopus stolonifer* with black sporangium and sporangiophore. Scale bars: A, D and F = 1 Cm; B, C, E, G, H and J = 20 µm

Table 1. Fungal Isolates of dragon fruit in each parts from Loei province

Fungi	Number of isolates		
	Stem	Flower	Fruit
Alternaria sp.	11	18	-
Bipolaris cactivora	-	30	14
Cladosporium cucumerinum	-	30	-
Colletotrichum gloeosporiodes	-	11	5
Fusarium sp.	-	21	-
Rhizopus stolonifer	-	-	3
Phomopsis sp.	4	-	-

Fruit rot caused by *Bipolaris cactivora*, morphology and epidemiology study

The most harmful species isolated from the fruits was *B. cactivora* causing rot symptoms around 4-7 days after harvesting and storage at room temperature (Figure 2) and conidia started germinating 4 hr after incubation (Figure 3). As *B. cactivora* was found in both flowers and fruits and proved as a serious pathogen of dragon fruit, it was then worthwhile to study the epidemiological aspect of this fungus. Regarding to the anamorph of *B. cactivora* on the fruit, conidiophores were pale to light brown, caespitose, straight or flexuous. Conidia with a basal hilum were straight, ellipsoidal, fusiform or obclavate, 2–4 septa, pale light brown to brown, and conidia size were (23.78-)25.30-47.30(-50.95) x (5.73-) 5.97-8.84(-9.84) µm. The results were similar to previous studies by Tarnowski *et al.* (2010) and Taba *et al.* (2007) (Table 2). Although, the size of conidia from this research had smaller conidia compare to previously reported by Ellis (1971) was 30 – 65 x 9 –12 µm (*Drechslera cactivora*) and Nakamura (1970) was 15 – 79 x 6 –15 µm (*Helminthosporium cactivora*) (22,23), this could be due to the variation of the pathogen.

Not only in Thailand, *B. cactivora* was also found as a pathogen related to cactus plants in other countries. Kim *et al*. (2004) assumed that *B. cactivora* it was associated with cactus stem rot in Korea (14, 15). Aside from the pathogenic impact to plants of *B. cactivora*, it was also claimed by the others Taba *et al*. (2007), Tarnowski *et al.* (2010), Ben-Ze'ev *et al*. (2011) and He *et al*. (2012) that *B. cactivora* is not a harmful pathogen in dragon fruit plants in different regions such as Japan, South Florida, Israel and Vietnam respectively (7,8,11,12) but this relation to the fruits from Loei province, the fungus was clearly pathogenic to the fruits.

Table 2. Comparison of the conidia sizes of *Bipolaris cactivora* and related pathogens compared with previous reports

Fungus	Conidia size (µm)	Morphology	References
B. cactivora	25.30-47.30 x 5.97-8.84	straight, ellipsoidal, fusiform or obclavate, 2–4 septa	This study
B. cactivora	24 – 51 x 9 – 13	pale-to-medium, golden brown, smooth and clavate with a protuberant hilum, 2-4 distoseptate	(8)
BPW-1 (*B. cactivora*)	35.3–45.5 x 8.5–10.9	Light brown to brown, porospores, straight,ellipsoidal, fusiform or clavate, 2–4 septa	(11)
Drechslera cactivora	30 – 65 x 9 –12	Mid golden brown, straight, ellipsoidal, fusiform or clavate 2–4 septa	(22)
Helminthosporium cactivora	15 – 79 x 6 –15	Dark brown,straight or slight or slightly curve, obtuse ellipsoidal	(23)

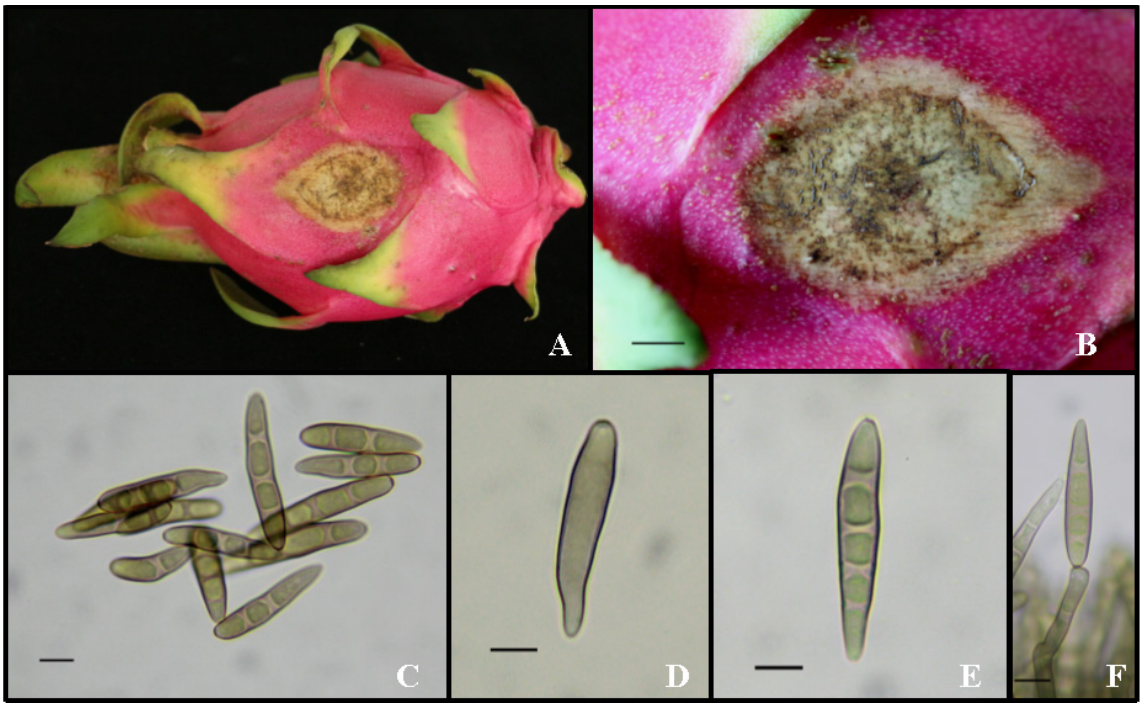

Figure 2. A-B: Rot symptom caused by *Bipolaris cactivora* on the dragon fruit, **C, E:** Conidia, **D:** Young spore without septa, **F:** Conidiophore with conidium attached. Scale bars: B = 1 cm; C– F = 10 µm

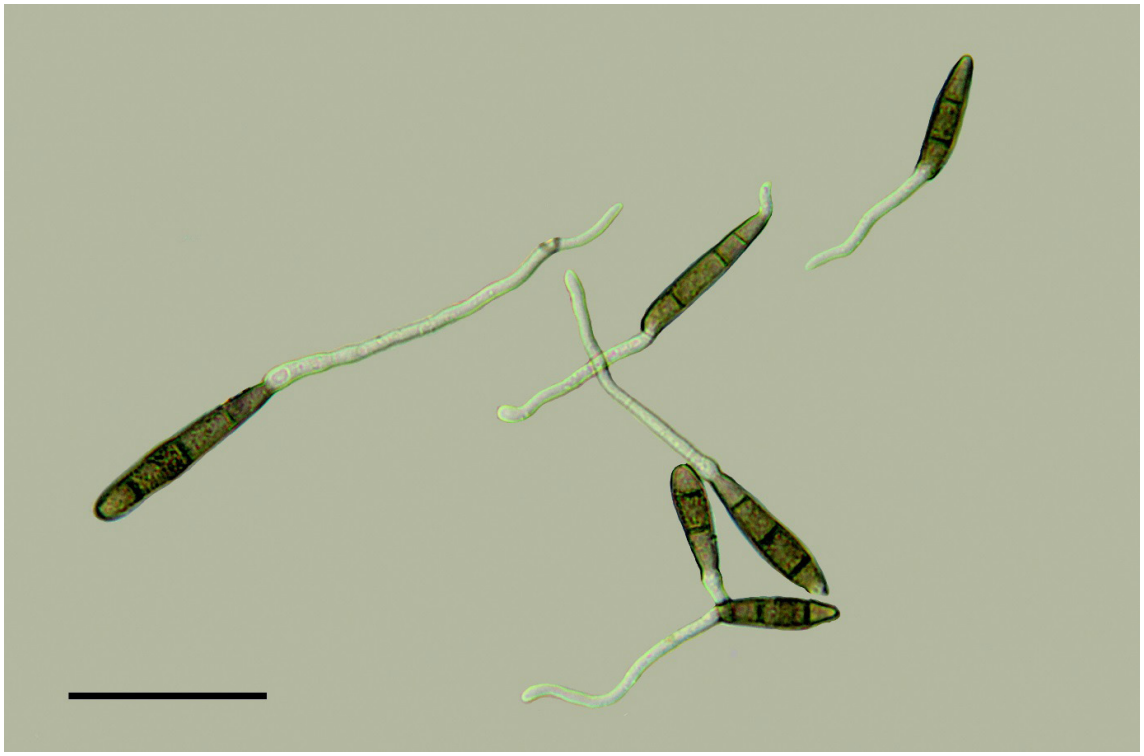

Figure 3. Conidial germination of *Bipolaris cactivora* at 4 hr, Scale bar 50 µm.

Incubation and latent period

Three isolates of *B. cactivora*, isolate 1 - 3 were able to cause the first symptom on the fruit surface at 24 h after inoculation at 25±1 °C but isolate 4 took 26 h to produce the first symptom (p value = 0.003). This pathogen started to produce a decent mass of conidia at hour 36 in isolate 1 and 2 but in isolate 3 and 4, it took more time to produce the conidia, 40 h on the surface of the fruits (p value < 0.01).

Lesion expansion

The diameter measurement of lesions on fruit surface caused by the fungus was achieved to evaluate the severity of the fungal isolates. The results suggested that among 4 isolates, isolate 2 was the fastest one according to growth rate, reaching 21.6 mm on the fruit at hour 42 after inoculation. In all isolates, the growth was sharply increased at hour 6 to 42. However, the growth rates of isolate 3 and 4 were slightly higher compared to isolate 1 and 2 i.e. *B. cactivora* could grow 3.8 mm/hr to infect the fruits (Figure 4). Meanwhile, on PDA, the pathogen could grow well, approximately 44 mm 55.62 mm at day 7 after inoculation (Figure 5). According to Bae *et al*. (2013), *B. cactivora* could develop mycelium on PDA about 32.3 mm after inoculation for 7 days (24) and Koo *et al* (2004) studied *B. spicifera.* They found that it could grow on media (PDA) around 80-90 mm within 7 days (25).

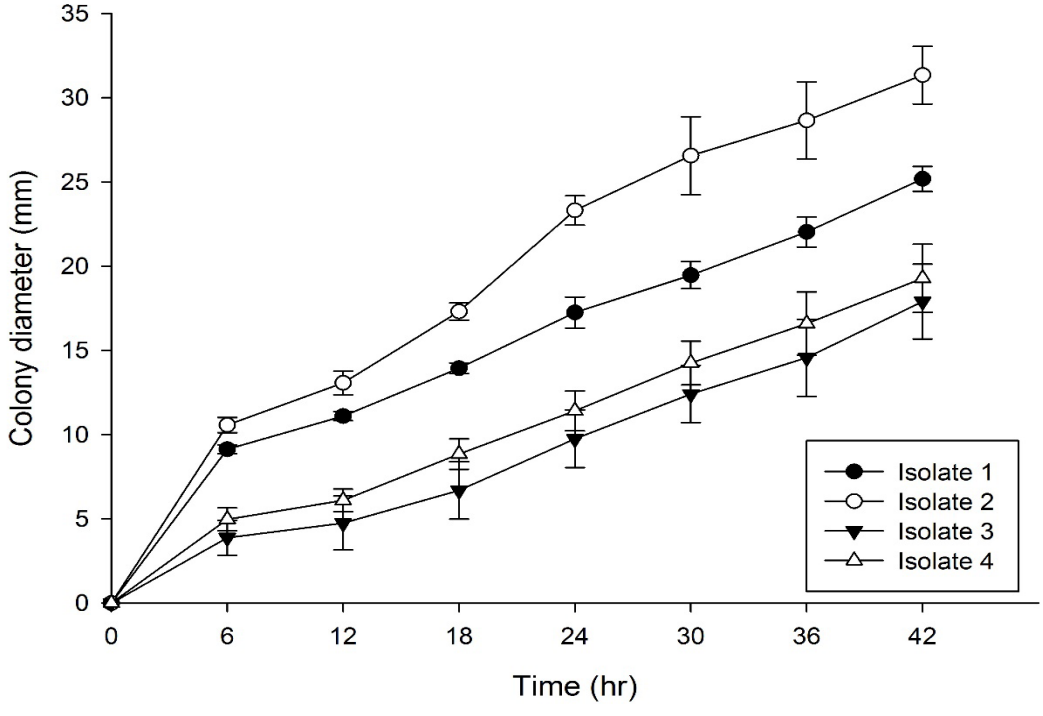

Figure 4. Mycelial growth rates of *Bipolaris cactivora* on the fruit among 4 fungal isolates within 42 hr (mean ± SD)

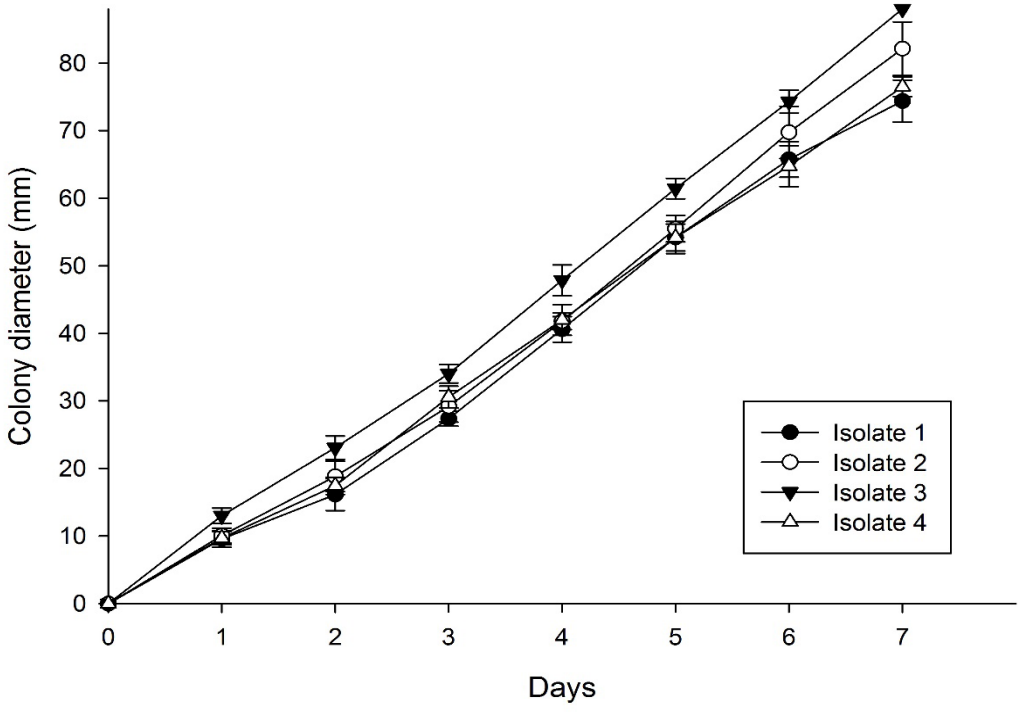

Figure 5. Mycelial growth rates of *Bipolaris cactivora* 4 fungal isolates on PDA within 7 days (mean ± SD)

Sporulation ability

Conidia were well produced by isolate 1 and 3 approximately 85-89 conidia per mm^2. In contrast, isolate 2 and 4 were found to produce in the lower number of conidia which was about 28-63 conidia per mm^2. However, no significant difference in conidia production in all isolates was indicated (p value = 0.518) (Figure 6). The impact of conidial production was, apart from fungal isolates, depending on culture media. *B. spicifera* produced a good number of conidia on V-8 medium followed by oat meal agar and potato dextrose agar, 119.3, 75.7 and 19.3 x 10^4 conidia/cm^2 respectively (25). This explained that using different species and media could yield the conidia numbers differently. In this report, *B. cactivora* could only produce a small proportion of the spores on PDA.

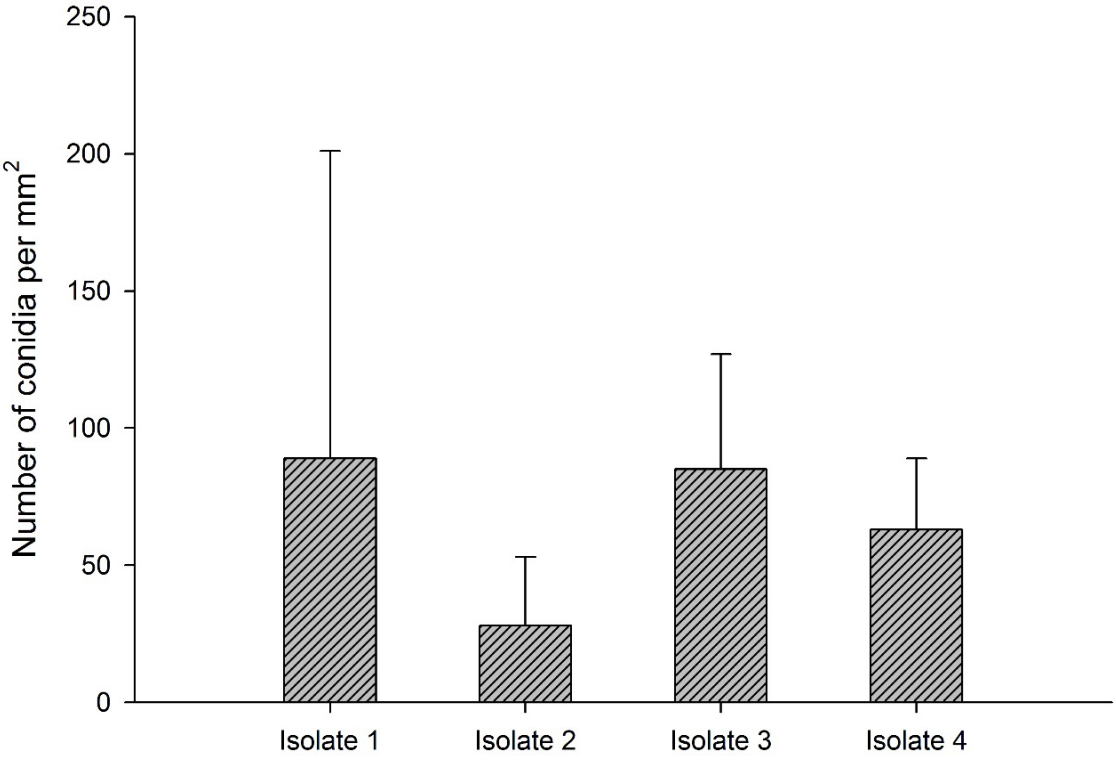

Figure 6. Numbers of *Bipolaris cactivora* conidia produced on the fruits (mean ± SD) which were not significantly different in all 4 isolates, p =0.518.

Several factors are influential to the pathogen to progress its life cycle e.g. humidity, temperature, plant varieties and pathogenicity of the fungus. Due to United States department of agriculture (USDA), it showed that *B. cactivora* is able to survive in various cactus varieties where the main source of primary inoculum is harbored (26). Weeds and other grass species are considered as the secondary source of inoculum because the pathogen is in conidial form until the conditions for germination are optimal to cause the disease in the host plants (27). In this study, *B. cactivora* was isolated only from flowers and fruits. This could be because of precipitation, rainfall, wind, high relative humidity and average temperature below 25°C that suited the conidia dispersal because Loei province is also located on a windy highland with altitude at 1,000 feet (304 m approximately) (28). In general, the initial of flowering occurs after raining season (30) and flowering period of the dragon fruit in Loei province is usually from May to June and can extend until October, which could allow the fungal conidia able to reside in the flowers until the fruits developed. The flowers generally open between 6.30-7:00 PM and extend until 10:00 PM to complete the opening, at about after midnight 2.00 AM the flower closes then begins to wilt (31). According to this time, the conidia are assumingly released and dispersed into the flowers but do not cause any infection. Once the fruits are

fully ripened, the fungal conidia that were once inside flower start germinating into mycelia which latterly exhibited the symptoms led by the fungus. Additionally, the land in the dragon fruit orchard was covered by weeds and grasses. This could possibly be another source of fungal inoculum, not only cacti. However, the first origin of the primary inoculum around the area has not been observed yet which is suggested to be the further study in order to seek for the complete life cycle of the fungus in the field.

4. Conclusion

According to the results, it showed that *B. cactivora* was the causal pathogen of flower and fruit rot of dragon fruit. The fungus was also found in flowers, which were the place of fungal inoculum leading to the rot disease in post-harvest dragon fruits collected from Loei province.

5. Suggestion

To confirm that *B. cactivora* from the rotten flowers was the inoculum causing the rot disease in fruits, the fungus from the flowers should be isolated and re-inoculated onto the healthy fruits to observe the disease development.

6. Acknowledgement

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